Effects of Phospholipid Unsaturation on the Membrane/Water Interface: A Molecular Simulation Study

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ABSTRACT Molecular dynamics (MD) simulations of fully hydrated bilayers in the liquid-crystalline state made of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) or 1-palmitoyl-2-elaidoyl-phosphatidylcholine (PEPC) were carried out to investigate the effect of the incorporation of a double bond in the phosphatidylcholine (PC) β-chain (*cis* or *trans*) on the membrane/water interface. The bilayers reached thermal equilibrium after 3 and 1 ns of MD simulations, respectively, and productive runs were carried out for 3 ns for each bilayer. As reference systems, the 1,2-dimyristoyl-phosphatidylcholine (DMPC) bilayer (M. Pasenkiewicz-Gierula, Y. Takaoka, H. Miyagawa, K. Kitamura, and A. Kusumi, 1999, *Biophys. J.* 76:1228–1240) and DMPC-cholesterol (Chol) bilayer containing 22 mol % Chol (M. Pasenkiewicz-Gierula, T. Róg, K. Kitamura, A. and Kusumi, 2000, *Biophys. J.* 78:1376–1389) were used. The study shows that at the interface of POPC, PEPC, and DMPC-Chol bilayers, average numbers of PC-water and PC-PC interactions are similar and, respectively, greater and smaller than in the DMPC bilayer. The average area/PC in mono-unsaturated bilayers is $\sim$4 Å^2 larger than in the DMPC bilayer; nevertheless, a strong correlation was found between a single molecular area (SMA) of a PC and the number of interactions this PC makes; i.e., PCs (either saturated or unsaturated) with the same SMA form similar numbers of intermolecular links. The numbers and corresponding SMAs are distributed about averages pertinent to each bilayer. No significant difference between *cis* and *trans* bonds was found.

INTRODUCTION

In a series of molecular dynamics (MD) simulation studies of the fully hydrated liquid-crystalline 1,2-dimyristoyl-phosphatidylcholine (DMPC) bilayer (Pasenkiewicz-Gierula et al., 1997, 1999) and DMPC-cholesterol (DMPC-Chol) (Pasenkiewicz-Gierula et al., 2000) bilayer containing 22 mol % Chol, we focused on the properties of the membrane/water interface. Particularly, we have investigated 1) hydrogen (H) bonding of water to DMPC and Chol, 2) interaction between water and the DMPC choline group (clathrated water), and 3) lipid-lipid interactions via water bridges and charge pairs. A water bridge is formed by a water molecule that is simultaneously H bonded to oxygen atoms of two lipid molecules (two phosphatidylcholines (PCs), PC and Chol, or two Chols). A charge pair is formed between a negatively charged oxygen atom of a lipid molecule (a nonester phosphate or carbonyl oxygen atom of PC or hydroxyl oxygen atom of Chol) and a methyl group of the positively charged choline (N-CH_3) moiety of neighboring PC. In both DMPC and DMPC-Chol membranes, extended networks of interlipid links via water bridges and charge pairs were found. These networks involve over 97% of the bilayer molecules. However, in the DMPC-Chol bilayer, the network is less branched as the number of the interlipid links/lipid is $\sim$30% smaller than in the DMPC bilayer, and the polar group region contains a greater number of water molecules. These are probably due to increased DMPC-DMPC distances in the DMPC-Chol bilayer as compared with the DMPC bilayer.

The primary aim of the present MD simulation study was to determine the effect of PC mono-unsaturation as well as the conformation (*cis* or *trans*) of the double bond in the β-chain on the organization of the membrane/water interface (the effect of the double-bond conformation on the alkyl chain region in mono-unsaturated PC bilayers will be published elsewhere). For this purpose, two PC membranes were built, one consisting of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and the other of 1-palmitoyl-2-elaidoyl-phosphatidylcholine (PEPC) molecules. Both POPC and PEPC molecules have the same palmitoyl (P) γ-chains (fully saturated, consisting of 16 carbon atoms), whereas their β-chains (mono-unsaturated, consisting of 18 carbon atoms) differ in the conformation of the double bond between C9 and C10 (cf. Fig. 1). In POPC, the double bond is in *cis* conformation (oleoyl (O) chain), and in PEPC, the double bond is in *trans* conformation (elaidoyl (E) chain).

Phospholipids with two asymmetric hydrocarbon chains, of which one is fully saturated in the γ position and the other is mono-*cis*- or poly-*cis*-unsaturated in the β position, are the most common in nature (Small, 1998). Among mono-*cis*-unsaturated PCs, POPC is the most abundant. Phospholipids with *trans*-unsaturated hydrocarbon chains are rare in nature; in a larger amount they have been found in some bacterial membranes at elevated temperatures (Okuyama et al., 1991) and in photosynthetic membranes of eukaryotic organisms (Dubertret et al., 1994). However, their role there has not been satisfactorily explained yet.

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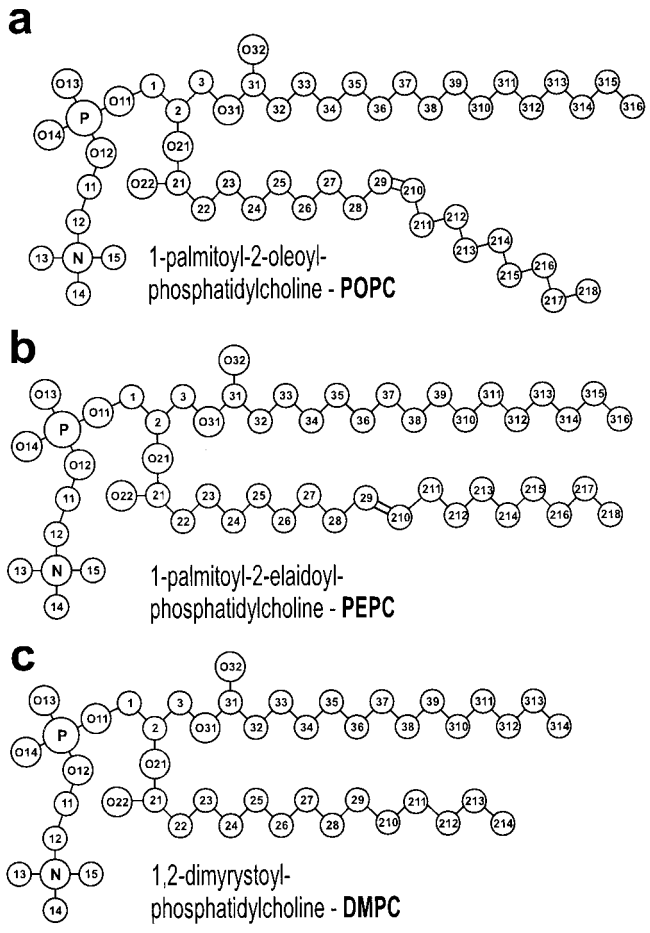

FIGURE 1 Molecular structures with numbering of atoms of POPC (*a*), PEPC (*b*), and DMPC (*c*) (chemical symbol for carbon atoms, C, is omitted).

Experimental studies show that in the membrane, a double bond in *cis* conformation interferes with hydrocarbon chain packing and destroys the cooperativity of the chain interactions in the bilayer (Kaneko et al., 1998; Di and Small, 1995; Davis, 1983; Stubbs and Smith, 1984). This substantially lowers the main phase transition temperature of chains with *cis*-double bonds located near the middle of the chain (Stubbs and Smith, 1984; Seelig and Waespe-Šarčević, 1978). The effect of a *trans* double bond on the hydrocarbon chain main phase transition temperature is weaker (Stubbs and Smith, 1984). The presence of a double bond in the alkyl chain increases the lateral PC-PC spacing in the bilayer (Stubbs and Smith, 1984). Order and reorientational motion of hydrocarbon chains in saturated, *cis*- and *trans*-unsaturated model membranes are similar (Kusumi et al., 1986; Subczynski and Wisniewska, 1996), whereas the translational diffusion of lipids (Kusumi et al., 1986) as well as small lipid-soluble molecules (Subczynski et al., 1989, 1990) is significantly lower in *cis*- and *trans*-unsaturated bilayers than in saturated ones. An introduction of a double bond into the alkyl chain lowers water penetration of the bilayer; the effect is greater for *cis*-unsaturated than *trans*-unsaturated bilayers (Subczynski et al., 1994). Cholesterol mixes at certain ratios with saturated and *trans*-unsaturated phospholipids whereas it is segregated out in *cis*-unsaturated bilayers (Subczynski et al., 1990; Pasenkiewicz-Gierula et al., 1991).

Computer simulation studies of bilayers made of unsaturated PCs complement experimental studies (Pearce and Harvey, 1993; Heller et al., 1993; Bolterauer and Heller, 1996; Huang et al., 1994; Feller et al., 1997; Hyvönen et al., 1997; Armen et al., 1998; Murzyn et al., 1999). A Langevin dynamics simulation of POPC, PEPC, and 1-palmitoyl-2-isolineleoyl-phosphatidylcholine in membrane environments represented by a mean field showed that structural and dynamic properties of PCs with a *trans* double bond are similar to those of saturated PCs, whereas PCs with a *cis* double bond behave differently (Pearce and Harvey, 1993). A 300-ps molecular dynamics (MD) simulation of a fully hydrated POPC bilayer consisting of 200 POPC and 5483 water molecules (~27,000 atoms) generated a system of characteristics similar to those obtained experimentally (Heller et al., 1993; Bolterauer and Heller, 1996). Using MD simulation, Hyvönen et al. (1997) showed that the vertical positions of the double bonds in a 1-palmitoyl-2-linoleoyl-phosphatidylcholine bilayer were widely distributed from nearly the center of the bilayer to the membrane/water interface. Recently, Feller et al. (1997) developed force-field parameters for unsaturated hydrocarbons and used them in an MD simulation study of a 1,2-dioleoyl-phosphatidylcholine (DOPC) bilayer at low hydration. Results of the simulation compared well with experimental results. An MD simulation of a hydrated POPC bilayer (Murzyn et al., 1999) showed that single C-C bonds next to the *cis* double bond in the β-chain were practically never in *gauche* conformations but had prevailing *trans* (180°) and *skew* ($\pm$120°) torsion angles, in agreement with experiments (Kaneko et al., 1998) and molecular mechanics calculations (Applegate and Glomset, 1991). The MD simulation additionally showed that these C-C bonds undergo rapid transitions between conformational states in the range between *skew* and *skew′* torsion angles (Murzyn et al., 1999). Torsion angles of C-C bonds next to the *trans* double bond in the β-chain of PEPC cover all range of angles from 0° to 360° and, as those in POPC, undergo rapid transitions between conformational states (Róg, 2000).

In the present research, results for POPC and PEPC bilayers (simulated for over 6 and 4 ns, respectively) are compared with those for DMPC and DMPC-Chol bilayers (simulated for over 6 ns and 5 ns, respectively) (Pasenkiewicz-Gierula et al., 1997, 1999, 2000). This comparison indicates that PC-water and PC-PC interactions in the interfacial region are similar in POPC, PEPC, and DMPC-Chol bilayers, but they differ from those in the DMPC bilayer. A correlation between the surface area occupied by a PC in the membrane and the readiness of the PC to interact with water and surrounding PCs was found.

MATERIALS AND METHODS

Simulation systems

Both POPC and PEPC membranes consisted of 72 (6 × 6 × 2) PC and 1922 water molecules; i.e., each bilayer contained ~27 water molecules per PC (~39% by weight). The experimentally determined numbers of water molecules per 1,2-dipalmitoyl-phosphatidylcholine (DPPC) and DOPC in fully hydrated bilayers range between 20 and 32.5 (Nagle and Wiener, 1988; Nagle 1993; Nagle et al., 1996, 1999; Tristram-Nagle et al., 1998; Ulrich et al., 1990, 1994). Because the number of water molecules per POPC and PEPC is likely to be between those for DPPC and DOPC (Rand and Parsegian, 1989), it is believed that 27 water molecules per POPC or PEPC were sufficient to fully hydrate them.

The initial structures of POPC and PEPC molecules were constructed on the basis of the minimized structures of DMPC A (Vanderkooi, 1991) obtained from x-ray diffraction data (Pearson and Pasher, 1979). The γ- and β-chains of POPC and PEPC are longer by two and four methylene groups, respectively, than the corresponding chains of DMPC. Besides, the β-chains of POPC and PEPC have double bonds between C9 and C10 (cf. Fig. 1). In POPC, the double bond is in the *cis* conformation (oleoyl (O) chain), and in PEPC, the double bond is in the *trans* conformation (elaidoyl (E) chain). Thus, to the DMPC γ- and β-chains two and four methylene groups, respectively, were added, and the hybridization of carbon atoms C9 and C10 in the β-chain (cf. Fig. 1) was changed to sp2. Then, the torsion angle of the POPC C9═C10 bond was set at 0° (*cis* conformation) and that of PEPC at 180° (*trans* conformation).

The initial structures of POPC and PEPC bilayers were obtained in the following way: 1) 36 PC molecules were randomly rotated around their long molecular axis (*z* axis); 2) the molecules were arranged in a 6 × 6 array in the bilayer *x*, *y* plane in a way to avoid van der Waals contacts (the initial *x*- and *y*-dimensions of both bilayers were 76 Å, giving an initial surface area per POPC and PEPC of ~160 Å^2); and 3) the second layer was obtained from the first one by applying P2 symmetry.

Simulation parameters

For PCs, optimized potentials for liquid simulations (OPLS) parameters (Jorgensen and Tirado-Rives, 1988), and for water, TIP3P parameters (Jorgensen et al., 1983) were used. The united-atom approximation was applied to CH, CH_2, and CH_3 groups of PCs. The atomic charges on POPC and PEPC were practically the same as those on DMPC (Pasenkiewicz-Gierula et al., 1999; Charifson et al., 1990). The parameters for the double-bonded carbon atoms in POPC and PEPC (cf. Fig. 1) not present in the original OPLS base were slightly modified parameters for the sp2 carbon atom in pyrimidines at positions 5 or 6. The procedure for supplementing the original OPLS base with the missing parameters for the PC headgroup was described by Pasenkiewicz-Gierula et al. (1999).

Simulation conditions

The POPC and PEPC bilayer membranes have been simulated for over 6 and 4 ns, respectively, using AMBER 4.0 (Pearlman et al., 1991). Three-dimensional periodic boundary conditions with the usual minimum image convention were used. The SHAKE algorithm (Ryckaert et al., 1977) was used to preserve the bond lengths of the water molecule, and the time step was set at 2 fs (Egberts et al., 1994). For nonbonded interactions, a residue-based cutoff was employed with a cutoff distance of 12 Å. To reduce calculation time of nonbonded interactions, each PC molecule (POPC and PEPC) was divided into six residues (residues 1–6 consisted of the following atoms and groups, respectively: 1, C218-C29; 2, C28-C23; 3, C22-C21 and O22; 4, O21, C1-C3, the phosphate group, the α-chain, and the choline group; 5, O31, O32 and C31-C34; and 6, C35-C316, cf. Fig. 1). Each residue was chosen in such a way that the total electrostatic charge on the residue was close to zero and the integrity of its chemical groups was preserved. The list of nonbonded pairs was updated every 25 steps.

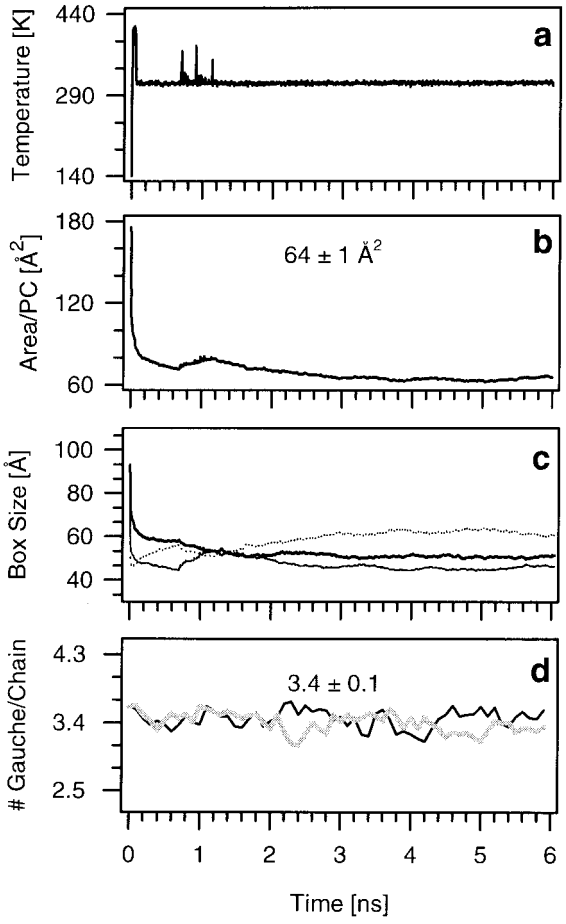

FIGURE 2 Diagrams showing the time development of the temperature (*a*), surface area per PC (*b*) (the equilibrium average surface area is 64 ± 1 Å^2), simulation box dimensions (*c*) (*x*, *thick line*; *y*, *thin line*; *z*, *light gray line*), and number of *gauche* bonds per O chain (*d*, *black line*), and P chain (*d*, *gray line*) (for both chains, the equilibrium average number is 3.4 ± 0.1) for the POPC membrane. The errors are standard deviations.

MD simulations were carried out at a constant pressure (1 atm) and temperature of 310 K (37°C), which is above the main phase transition temperature for a POPC bilayer (−5°C) (Seelig and Waespe-Šarčević, 1978) and a PEPC (26°C) (Seelig and Waespe-Šarčević, 1978) bilayer. Temperatures of the solute and solvent were controlled independently. Both the temperature and pressure of the systems were controlled by the Berendsen method (Berendsen et al., 1984). The relaxation times for temperatures and pressure were set at 0.4 and 0.6 ps, respectively. Applied pressure was controlled anisotropically, where each direction was treated independently and the trace of the pressure tensor was kept constant (1 atm).

RESULTS

Characterization of the membrane systems and comparison with experimental data

The approach to the thermally equilibrated state of the POPC bilayer membrane in the liquid-crystalline phase was

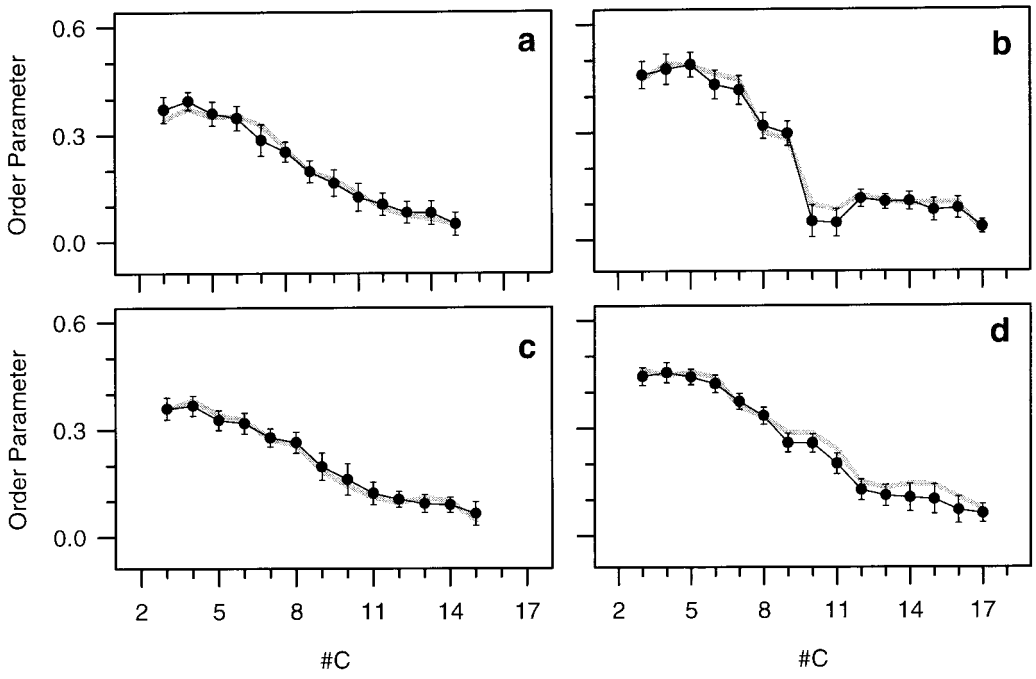

FIGURE 3 The molecular order parameter (S_{mol}) profiles calculated for the P chain of POPC (*a*), O chain of POPC (*b*), P chain of PEPC (*c*), and E chain of PEPC (*d*). A black line and solid dots represent averages over the productive runs (3.0–6.0 ns for the POPC bilayer and 1.0–4.0 ns for the PEPC bilayer); a gray line represents averages over the last 100 ps of the productive runs (5.9–6.0 ns for POPC bilayer and 3.9–4.0 ns for the PEPC bilayer). Error bars are standard deviations.

observed from the onset of simulation until 6000 ps by monitoring the following parameters of the system: the temperature (Fig. 2 *a*), average surface area per PC (Fig. 2 *b*), dimensions of the simulation box (Fig. 2 *c*), number of *gauche* conformations per β- and γ-chain (Fig. 2 *d*), and molecular order parameter (S_{mol}) profile (Fig. 3). Similar time profiles were obtained for the PEPC bilayer (data not shown). The average surface area per POPC reached a stable value of 64 $\pm$ 1 Å^2 after ~3.0 ns (errors of the surface area/PC, S_{mol}, number of *gauche* rotamers/chain, bilayer thickness, and lifetimes are given in standard deviations as in our previous papers (Pasenkiewicz-Gierula et al., 1997, 1999, 2000); errors of values in Fig. 8 and Tables 2–5 are given in standard errors). The number of *gauche* conformations per palmitoyl (P) and oleoyl (O) chains stabilized after ~1 ns at the level of 3.4 $\pm$ 0.1 for both chains. The S_{mol} profile converged after ~2.0 ns. Other parameters, like the temperature and potential energy stabilized in shorter time periods. In the PEPC bilayer membrane, the average surface area per PC of 64 $\pm$ 1 Å^2, the number of *gauche* conformations per P chain of 3.3 $\pm$ 0.1 and per elaidoyl (E) chain of 3.5 $\pm$ 0.1, as well as the order parameter profile stabilized well within 1.0 ns. Thus, it was concluded that POPC and PEPC bilayers had reached thermal equilibrium after 3.0 ns and 1.0 ns, respectively.

The average surface area per POPC in the bilayer of 64 $\pm$ 1 Å^2 is between values of 63 Å^2 (Smaby et al., 1997) and 66 Å^2 (Hyslop et al., 1990) estimated for a POPC bilayer from POPC monolayer studies. In those studies, the mean molecular area/POPC in the bilayer was assumed to be equal to that in the monolayer at a surface pressure of 30 mN/m (Smaby et al., 1997) and 20 mN/m (Hyslop et al., 1990). Experimental estimates of the average surface area per PEPC in the bilayer are not available; however, the same values of the area/PC in the POPC and PEPC bilayers seem reasonable. Experimentally measured numbers of *gauche* rotamers per P chain of DPPC in the bilayer at temperatures above the main phase transition temperature of 41°C, are 3.0–6.0 (Seelig and Seelig, 1974), 3.8 (Nagle and Wilkinson, 1978), and 3.6–4.2 (Mendelsohn et al., 1989). In the simulated POPC and PEPC membranes at 37°C, numbers of *gauche* rotamers per P chain of POPC and PEPC, are 3.4 and 3.3, respectively, and thus, they are in reasonable agreement with experimentally determined values. The calculated S_{mol} profiles for the P and O chain of POPC and the P and E chain of PEPC (Fig. 3) are similar to those obtained by Seelig and Seelig (1977) for the P chain of POPC, and Seelig and Waespe-Šarčević (1978) for the O and E chain of POPC and PEPC, respectively.

The equilibrium electron density profile across the POPC bilayer (along the *z* axis) shown in Fig. 4, has a similar shape to that obtained by reconstruction of experimental data for DPPC bilayers (Nagle et al., 1996). Because there is no firm definition of the bilayer thickness (Nagle et al., 1996) we calculated three parameters related to this quantity: 1) an average P-P spacing (distance between average

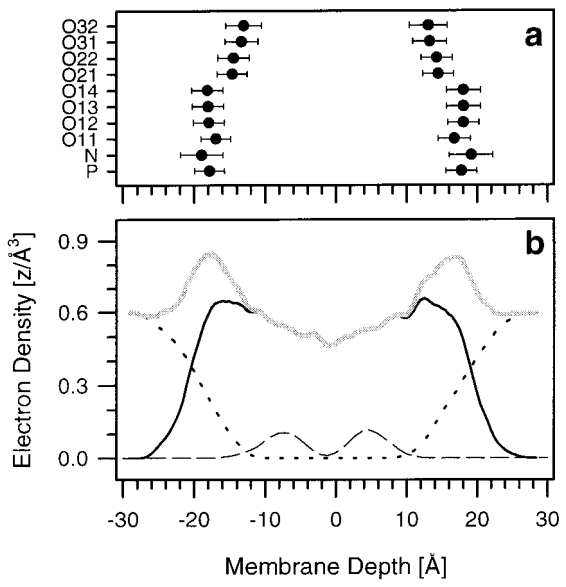

FIGURE 4 (*a*) Average positions and distributions of the oxygen (O), nitrogen (N), and phosphorus (P) atoms (cf. Fig. 1) along the bilayer normal in the POPC bilayer; (*b*) Equilibrium electron density profiles along the bilayer normal of the lipids (*solid black line*), water (*dotted line*), lipids and water (*thick gray line*), and the carbon atoms forming the double bond (C9 and C10) (*dashed line*) in the POPC bilayer.

positions of P atoms in two leaflets of the bilayer), 2) an average N-N spacing, and 3) a pick-to-pick distance in the electron density profile across the bilayer. The three values are as follows: for the POPC bilayer, 35.5 ± 0.2, 37.6 ± 0.2 Å, and 28.5 ± 2 Å, respectively; for the PEPC bilayer, 36.0 ± 0.1, 38.2 ± 0.1 Å, and 29.0 ± 2 Å, respectively; and for the DMPC bilayer, 32.9 ± 0.1 Å, 35.1 ± 0.1 Å, and 26.5 ± 2 Å, respectively. These values show a proper trend as bilayers built of PCs with longer chains should be thicker. Such a trend was not followed in the case of a DOPC bilayer whose thickness at 30°C was smaller (35.3 Å) (Tristram-Nagle et al., 1998) than that of DPPC bilayer at 50°C (36.4 Å) (Nagle et al., 1996); however, DOPC has two mono-unsaturated chains and a surprisingly large surface area/PC of ~70 Å^2 (Tristram-Nagle et al., 1998).

The results summarized above suggest that the simulated POPC and PEPC bilayers obtained here reproduce various properties of mono-unsaturated PC bilayers in the liquid-crystalline phase that have been measured experimentally. Therefore, it is concluded that the simulated bilayers provide good models for POPC and PEPC membranes.

In the present report, in analyzing 3-ns trajectories generated in MD simulations of the well-equilibrated POPC (between 3 and 6 ns) and PEPC (between 1 and 4 ns) bilayers, we concentrate on the effect of the double bond (*cis* or *trans*) on the properties of the membrane/water interface. In particular, we pay special attention to how the double bond and its conformation affect interactions between PC polar groups and water and the formation of PC-PC water bridges and charge pairs.

In this study, we used the same geometrical definitions of H bonding, water bridging, charge pairing, and their lifetimes as well as that of the nearest-neighbor water as in our previous papers (Pasenkiewicz-Gierula et al., 1997, 1999). Although a simple TIP3P potential for water was used, simulation reproduced well both average numbers of water-water and PC-water H bonds and the H bond geometry (cf. Pasenkiewicz-Gierula et al., 1997).

1) An H bond between an OH group of a water molecule and an oxygen atom of PC is judged to be formed when the O···O distance (r) is ≤3.25 Å and the angle θ between the O···O vector and the OH bond (the O···O-H angle) is ≤35°. The distance of 3.25 Å is the position of the first minimum in the radial distribution function (RDF) of the water oxygen atoms (Ow) relative to an oxygen atom of PC.

2) A water bridge is made by a water molecule that is simultaneously H bonded to two lipid oxygen atoms. We distinguish between intermolecular and intramolecular water bridges.

3) A charge pair is formed between a positively charged choline methyl group (N-CH_3) and negatively charged non-ester phosphate (Op) or carbonyl oxygen (Oc) atoms when they are located within 4.0 Å from each other. We distinguish between intermolecular and intramolecular charge pairs.

4) PC-PC association via H bonding and charge pairing is a dynamic state. To calculate its lifetime, a history of each PC-PC pair was monitored every 1.0 ps for the time from its first appearance (after equilibration of the system) until the final time of MD simulation. In this analysis, if the association was temporarily broken but re-formed within 60 ps between the same molecules, the break was ignored, whereas a break longer than 60 ps was treated as the final decay. The time of the binding between successive breaks is called a firm bonding time, whereas the time of a break is called a temporary break time (if it is shorter than 60 ps).

5) A nearest-neighbor water molecule of a PC oxygen atom (N-CH_3) is defined as any water molecule whose oxygen atom is within 3.25 Å (4.75 Å) from the atom (N-CH_3). 4.75 Å is the position of the first minimum in RDF of the Ows relative to a N-CH_3. When counting nearest-neighbor water molecules to a N-CH_3, the water molecules that are simultaneously H bonded to any PC oxygen atom or are nearest neighbor of another N-CH_3, are excluded.

Water-POPC and water-PEPC interactions

Interactions between water and polar groups of PCs in POPC and PEPC bilayers are compared with those in DMPC and DMPC-Chol bilayers. The DMPC and DMPC-Chol bilayers were simulated for over 6 ns and 5 ns, respectively, and the results of the simulations are described in our previous papers (Pasenkiewicz-Gierula et al., 1997, 1999, 2000).

TABLE 1 Average numbers of nearest-neighbor (n.n.) water molecules, H bonds, and H bonds involved in water bridges (intra- and intermolecular) per PC oxygen atom (cf. Fig. 1) as well as the percentage of H bonds formed on each PC oxygen atom in POPC, PEPC, DMPC-Chol, and DMPC bilayers

	Bilayer	O14	O13	O22	O32	O11	O12	Total
No. n.n. water molecules	POPC	2.2	2.2	0.8	0.5	0.5	0.3	6.5
	PEPC	2.2	2.2	0.8	0.5	0.5	0.3	6.5
	DMPC-Chol*	2.3	2.2	0.7	0.6	0.5	0.3	6.6
	DMPC†	2.1	2.1	0.6	0.6	0.4	0.3	6.1
No. H bonds	POPC	2.1	2.1	0.6	0.4	0.4	0.1	5.7
	PEPC	2.1	2.1	0.6	0.4	0.4	0.1	5.7
	DMPC-Chol*	2.1	2.1	0.5	0.5	0.4	0.1	5.7
	DMPC†	2.0	2.0	0.5	0.4	0.3	0.1	5.4
% H bonds formed on each oxygen	POPC	37	37	11	7	7	2	100
	PEPC	37	37	11	7	7	2	100
	DMPC-Chol*	37	37	9	9	7	2	100
	DMPC†	38	38	9	8	6	2	100
No. H bonds involved in water bridging	POPC	0.51	0.52	0.16	0.12	0.11	0.03	1.45
	PEPC	0.52	0.52	0.17	0.11	0.11	0.03	1.46
	DMPC-Chol*							
	DMPC†	0.57	0.59	0.22	0.12	0.12	0.04	1.66
No. H bonds involved in intermolecular bridge	POPC	0.45	0.45	0.09	0.09	0.09	0.01	1.18
	PEPC	0.45	0.45	0.08	0.09	0.09	0.01	1.17
	DMPC-Chol*							
	DMPC†	0.51	0.51	0.11	0.10	0.10	0.03	1.36
No. H bonds involved in intramolecular bridge	POPC	0.07	0.07	0.07	0.03	0.02	0.02	0.29
	PEPC	0.06	0.07	0.09	0.02	0.02	0.02	0.29
	DMPC-Chol*							
	DMPC†	0.06	0.08	0.11	0.02	0.02	0.01	0.30

*Pasenkiewicz-Gierula et al. (2000).
†Pasenkiewicz-Gierula et al. (1997).

Interactions between water and PC oxygen atoms

Average numbers of H bonds formed between water molecules and oxygen atoms of PC in POPC and PEPC membranes as well as numbers of H-bonded and nearest-neighbor (n.n.) water molecules are given in Tables 1 and 2 and compared with those in DMPC and DMPC-Chol membranes (Pasenkiewicz-Gierula et al., 1997, 2000). In agreement with experimental results (Arnold et al., 1983; Nagle, 1993), the average number of H-bonded water molecules per PC is, depending on the PC, between 5.0 and 4.5. The geometry of H bonds in monounsaturated bilayers is the same as that in the fully saturated one (Pasenkiewicz-Gierula et al., 1997). The percentage of Ops forming two (61 ± 1%) and three (23 ± 1%) H bonds with water is higher in POPC and PEPC bilayers than in the DMPC bilayer (58 ± 1% and 21 ± 1%, respectively (Pasenkiewicz-Gierula et al., 1997)). It is interesting to note a higher asymmetry in H bonding between the β- and γ-chain carbonyl oxygen atoms of POPC and PEPC than DMPC (Table 1). An example of a POPC phosphate group with six n.n. water molecules, of which five are H bonded, is shown in Fig. 5 *a*.

Interaction between water and the choline group of PC

Numbers of the choline n.n. water molecules in POPC, PEPC, DMPC-Chol, and DMPC bilayers are given in Table 2. These molecules form clathrate-like structures around choline groups (Alper et al., 1993; Damodaran and Merz, 1994; Perera et al., 1996; Pasenkiewicz-Gierula et al., 1997). The geometry of water molecules forming clathrates (Pasenkiewicz-Gierula et al., 1997) is the same in all membranes. An example of water molecules forming a clathrate-like structure around a POPC choline group is shown in Fig. 5 *b*.

PC-PC associations via water bridges and charge pairs

Our previous MD simulation studies revealed that at the membrane/water interface of both DMPC and DMPC-Chol

TABLE 2 Average numbers of H-bonded water molecules, water molecules in a clathrate, and the total number of bound water molecules per PC in POPC, PEPC, DMPC-Chol, and DMPC bilayers

Bilayer	Number of H-bonded water molecules	Number of clathrated water molecules	Number of PC-bound water molecules
POPC	5.0 ± 0.04	6.6 ± 0.03	11.6 ± 0.1
PEPC	5.0 ± 0.04	6.6 ± 0.03	11.6 ± 0.1
DMPC-Chol*	4.8 ± 0.04	6.5 ± 0.03	11.3 ± 0.1
DMPC†	4.5 ± 0.04	6.1 ± 0.03	10.6 ± 0.1

The errors are standard errors.
*Pasenkiewicz-Gierula et al. (2000).
†Pasenkiewicz-Gierula et al. (1997).

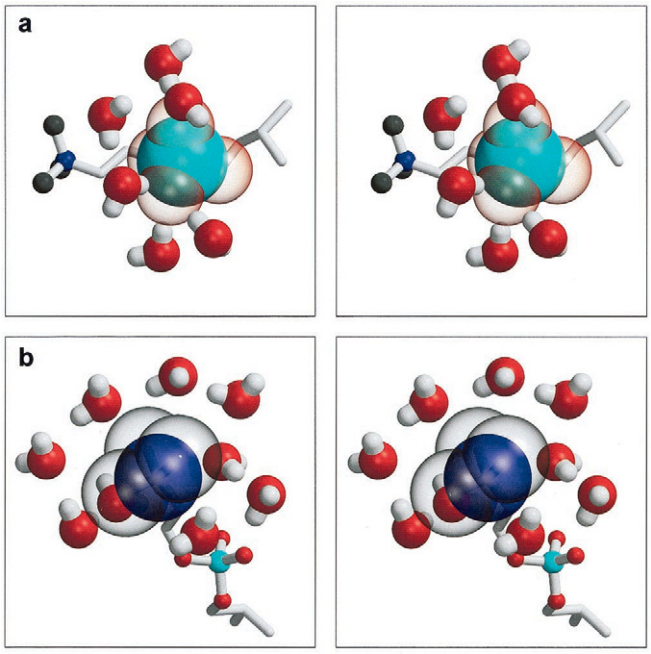

FIGURE 5 Stereo views of hydration of an arbitrary chosen POPC headgroup in the membrane. The nearest-neighbor water molecules of the POPC nonester phosphate oxygen atoms (*a*) (five of the six water molecules are H bonded) and choline methyl groups (N-CH_3) (*b*) (several of the water molecules form a clathrate-like structure). The water molecules are shown in standard colors as the CPK model. The phosphorus atom is in light blue (*a*) and the nitrogen atom is in dark blue (*b*); the phosphate oxygen atoms and N-CH_3 groups are transparent spheres. The image was produced with MolScript (Kraulis, 1991) and Raster3D (Merritt and Bacon, 1997).

bilayer membranes, lipid molecules are linked with one another via water bridges and charge pairs (Pasenkiewicz-Gierula et al., 1997, 1999, 2000). In the present study, the formation of water bridges and charge pairs among PC molecules in POPC and PEPC bilayers is investigated.

PC-PC interactions via water bridges

Average numbers of intermolecular water bridges per PC in POPC, PEPC, and DMPC-Chol bilayers are similar and smaller than that in the DMPC bilayer (Table 3; cf. Fig. 6). Average numbers of intramolecular water bridges per PC in POPC, PEPC, and DMPC-Chol bilayers are also similar, but they are larger than that in the DMPC bilayer (Table 3). Intramolecular water bridges constitute ~30% of all bridges in POPC, PEPC, and DMPC-Chol bilayers and 20% of all bridges in DMPC bilayer.

Like in the DMPC bilayer (Pasenkiewicz-Gierula et al., 1997), intermolecular water bridges in POPC and PEPC bilayers involve Op in 81% and Oc in 19%, whereas intramolecular water bridges involve Op in 56% and Oc in 44%. Thus, the most frequent intermolecular and intramolecular bridges are Op···HOH···Op and Op···HOH···Oc, respectively. There is no significant difference in H-bonding geometry of bridging and nonbridging water.

Average lifetimes of individual intermolecular water bridges in POPC, PEPC, DMPC-Chol, and DMPC bilayers are given in Table 3. For a PC-PC association linked by water bridges (water exchange is allowed), due to the finite analysis times, only the lower limits of the average lifetimes can be estimated (for POPC, PEPC, and DMPC bilayers, the analysis time was 3 ns; for the DMPC-Chol bilayer, the analysis time was 2 ns). The estimated lifetimes are given in Table 3; however, because of different analysis times, their exact comparison is not possible. Nevertheless, in POPC and PEPC bilayers, 3% and 5% of the PC-PC links survived for the time longer than 3 ns, respectively, whereas in DMPC-Chol and DMPC bilayers 8% and 7.5% of the PC-PC links survived for the time longer than 2 ns and 3 ns, respectively (Table 3). Details concerning the lifetime analysis are described in Pasenkiewicz-Gierula et al. (1999).

PC-PC interactions via charge pairs

Like in the case of water bridges, average numbers of intermolecular charge pairs per PC in POPC, PEPC, and DMPC-Chol bilayers are similar and smaller than that in the DMPC bilayer (Table 4; cf. Fig. 6). However, the numbers of intramolecular charge pairs per PC in the four bilayers do not show any clear trend; in the PEPC bilayer, the number is the largest, and in the POPC bilayer it is the smallest (Table 4). Intramolecular charge pairs constitute from 11–16% of all charge pairs made in these bilayers (Table 4).

Average lifetimes of individual charge pairs for POPC, PEPC, DMPC-Chol, and DMPC membranes are given in Table 4 as well as lower limits of the average lifetimes of PC-PC associations linked by charge pairs. In POPC and PEPC bilayers, 19% and 10% of the PC-PC links survived for the time longer than 3 ns, respectively, whereas in DMPC-Chol and DMPC bilayers 19% and 18% of the PC-PC links survived for the time longer than 2 ns and 3 ns, respectively (Table 4).

PC-PC interactions via charge pairs and/or water bridges

On average, PC molecules in the DMPC bilayer make 5.3 individual intermolecular links via water bridges and charge pairs whereas those in POPC, PEPC, and DMPC-Chol bilayers make 4.2–4.4 links/PC (the maximum number of simultaneous links per PC was 18). As some of the water bridges and charge pairs are multiple, and some of the PCs are simultaneously connected by water bridges and charge pairs, each PC is, on average, linked with 2.2 other PCs (PC-PC pairs) in POPC, PEPC, and DMPC-Chol bilayers, and 2.7 PCs in the DMPC bilayer (Table 5) (the maximum number of simultaneous interlipid links per PC was 7). These links connect the majority of the membrane PCs at any instant. The lower limits of the average lifetimes of

TABLE 3 Inter- and intramolecular PC-PC water bridges and water-bridged pairs in POPC, PEPC, DMPC-Chol, and DMPC bilayers

Water bridge	Number/PC (% PCs)	Lifetime (ps)
POPC bilayer		
Op, Oc-Op, Oc	0.49 ± 0.01 bridges/POPC (inter)	30 ± 60
	68 ± 3% single, 32 ± 3% multiple	
	0.19 ± 0.01 bridges/POPC (intra)	
POPC-POPC	0.41 ± 0.01 pairs/POPC	680 ± 730*
	44 ± 1 POPC (62%)	(3% ≥ 3000 ps)
PEPC bilayer		
Op, Oc-Op, Oc	0.52 ± 0.01 bridges/PEPC (inter)	30 ± 60
	68 ± 3% single, 32 ± 3% multiple	
	0.21 ± 0.01 bridges/PEPC (intra)	
PEPC-PEPC	0.43 ± 0.01 pairs/PEPC	830 ± 890*
	47 ± 1 PEPC (65%)	(5% ≥ 3000 ps)
DMPC-Chol bilayer†		
Op, Oc-Op, Oc	0.50 ± 0.01 bridges/DMPC (inter)	27 ± 65
	69 ± 3% single, 31 ± 3% multiple	
	0.19 ± 0.01 bridges/DMPC (intra)	
DMPC-DMPC	0.43 ± 0.01 pairs/DMPC	530 ± 600*
	35.5 ± 1 DMPC (64%)	(8% ≥ 2000 ps)
DMPC bilayer‡		
Op, Oc-Op, Oc	0.57 ± 0.01 bridges/DMPC (inter)	50 ± 50
	61 ± 3% single, 39 ± 3% multiple	
	0.14 ± 0.01 bridges/DMPC (intra)	
DMPC-DMPC	0.55 ± 0.01 pairs/DMPC	730 ± 900*
	55 ± 1 DMPC (76%)	(7.5% ≥ 3000 ps)

Numbers of water bridges, bridged molecules (%), and the lifetimes of the water bridging are given (Op and Oc are nonester phosphate and carbonyl oxygen atoms, respectively). The errors are standard errors, except for the lifetimes, where errors are standard deviations (due to the method of lifetime calculations).

*Only lower-limit estimate; see text (in parentheses, the percentage of lifetimes longer than the time window used for analysis is given).

†Pasenkiewicz-Gierula et al. (2000).

‡Pasenkiewicz-Gierula et al. (1999).

PC-PC pairs in the membranes are given in Table 6, together with average times of firm bonding, temporary break times, and numbers and frequencies of breaks (cf. Pasenkiewicz-Gierula et al., 1999, and definitions above). In POPC and PEPC bilayers, 26% and 19% of the PC-PC links survived for the time longer than 3 ns, the time window of the present analysis, whereas in DMPC-Chol and DMPC bilayers, 27% and 24% of the PC-PC links survived for the time longer than 2 ns and 3 ns, respectively (Table 6).

Networks of PC-PC links in the membrane/water interface and a cluster size analysis

Networks of interlipid links in DMPC and POPC bilayers at 3.5 ns of the simulation time are shown in Fig. 6. Associations that lasted at least 60% of the 10-ps time window are considered stable and displayed in the figure. Such a treatment followed from the observation that PC-PC links via water bridges and charge pairs can temporarily break and the ratio of the sum of the firm bonding and temporary break times to the firm bonding time is ~10:6 (Pasenkiewicz-Gierula et al., 1999). As can be seen from Fig. 6, the majority of the PCs in both membranes are connected with one another and form extended clusters.

For one-lipid-type systems, i.e., POPC, PEPC, and DMPC bilayers, a cluster size analysis with a 10-ps time window indicated that all PC molecules in each bilayer formed one extended cluster except for one or two PC molecules. In POPC and PEPC bilayers, each PC remained in the linked state, on average, for 98% of the analysis time, and in the DMPC bilayer, for 99% of the analysis time. The dynamic nature of PC-PC links in the interfacial region of the membrane is apparent when a cluster size is analyzed with a 1-ps time window (the time step for all trajectories analyzed in this paper). Fig. 7, *a–c*, shows the percentage of PCs participating in the formation of clusters of given sizes via both mechanisms, exclusively via charge pairs, and exclusively via water bridges, respectively, in POPC, PEPC, and DMPC bilayers.

Correlation between the surface area/PC and the number of interlipid links/PC

Single molecular area

The results obtained so far indicate that average numbers of H bonds with water, water bridges, and charge pairs formed by PC molecules in the interfacial region of the bilayer are

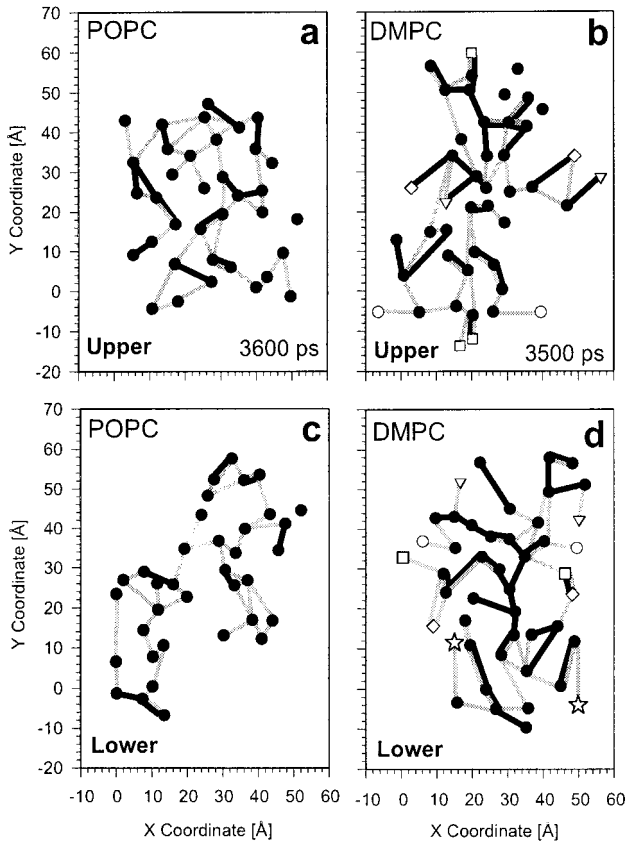

FIGURE 6 PC-PC cross-links via water bridges (*black*) and charge pairs (*gray*) for the upper (*a*) and lower (*c*) leaflet of the POPC bilayer at 3600 ps and for the upper (*b*) and lower (*d*) leaflet of the DMPC bilayer at 3500 ps. A black dot shows the location of the phosphorus atom of PC in the *x*, *y* plane. Pairs of open symbols represent images of "real" PC molecules, which are to show links between PCs of adjacent simulation boxes via periodic boundary conditions. Lipid links that lasted at least 60% of the time window of 10 ps are considered stable and shown here.

well correlated with the average surface area available to the PCs. The average surface area per PC is 64 ± 1 Å^2 in POPC and PEPC bilayers and 60.2 ± 1.0 Å^2 in the DMPC bilayer (Pasenkiewicz-Gierula et al., 1999). However, analyses of Shinoda and Okazaki (1998) and that shown in Fig. 8 *d* indicate that areas occupied by individual PC molecules in the membrane are broadly distributed. The intention of the analysis below is to determine whether numbers of intermolecular interactions formed by individual PCs are also distributed and correlated with the areas available to the PC headgroups. For this purpose, a surface area of each PC headgroup (a single molecular area, SMA) in the bilayer was determined by means of a modified two-dimensional Voronoi tessellation method (Bernal, 1959). This method was applied to one-type lipid bilayers, i.e., POPC, PEPC, and DMPC bilayers. A procedure similar to that of Shinoda and Okazaki (1998) was used, and the centers of masses of the PC headgroups in each bilayer leaflet were projected on the bilayer plane under the periodic boundary conditions. The positions of the maxima in the SMA distributions obtained for the bilayers corresponded well to average molecular areas calculated for these bilayers from the *x*- and *y*-dimension of the simulated boxes and given above (Fig. 8 *d*). Similar distributions and averages were obtained when the SMA of the whole PC, instead of the headgroup, was calculated.

Number of intermolecular links made by an individual PC molecule

Hydration of a PC molecule might be described in terms of the number of water molecules that are H bonded to the PC. With increasing SMA, the hydration may increase in two ways: 1) the number of H bonds formed by each oxygen atom of the PC remains the same and only the number of water molecules H bonded to the PC increases and 2) the number of both H bonds and H-bonded water molecules increases. In the former case, simple H bonds take the place of the broken bridging H bonds. In the latter case, new H bonds are formed. In the analysis below, changes in the number of PC H bonds with water (and not in the number of H-bonded water molecules) with increasing SMA were monitored.

In Fig. 8, numbers of H bonds with water/PC (Fig. 8 *a*), water bridges/PC (Fig. 8 *b*), and charge pairs/PC (Fig. 8 *c*) as functions of SMA are shown for POPC, PEPC, and DMPC bilayers. The numbers were obtained by averaging over molecules with the same SMA and the productive simulation time of 3 ns. Errors are given in standard errors. Meaningful results were obtained for SMA between 50 and 80 Å^2; for smaller and larger SMA, the statistics are poor. As expected, with increasing SMA, the number of H bonds with water/PC increases and numbers of charge pairs and water bridges per PC decrease. However, a greater than twofold increase in SMA (from 40 Å^2 to 90 Å^2) results in only ~30% increase in the number of H bonds with water/PC (from 4.4 to 5.6), whereas it results in a greater than twofold decrease in the number of water bridges/PC (from 1.3 to ~0.6). The decrease in the number of charge pairs is less regular (see below). An increase in the hydration of PC headgroups with increasing SMA is limited by the ability of the PC oxygen atoms to form H bonds with water and by the accessibility of the carbonyl oxygen atoms to water molecules. With increasing SMA from 40 to 80 Å^2, the number of H bonds formed on the carbonyl oxygen atoms increases by 31%, whereas that of the phosphate oxygen atoms increases by 14%. This indicates that even for small SMA, Ops are nearly fully hydrated. A strong correlation between SMA and the number of charge pairs/PC is observed for SMA larger than 50 Å^2 (Fig. 8 *c*).

When SMA is less than 55 Å^2, there are evident disparities among the bilayers. Most likely, they are caused by different accessibility of carbonyl oxygen atoms to N-CH_3 for small SMA values in POPC and PEPC bilayers as

TABLE 4 Inter- and intramolecular PC-PC charge pairs in POPC, PEPC, DMPC-Chol, and DMPC bilayers

Charge pair	Number/PC (% PCs)	Lifetime (ps)
POPC bilayer		
Op, Oc-N-CH_3	1.71 ± 0.04 pairs/POPC (inter)	150 ± 250
	25 ± 3% single, 75 ± 3% multiple	
	0.21 ± 0.01 pairs/POPC (intra)	
POPC-POPC	0.88 ± 0.01 pairs/POPC	1400 ± 1000*
	65 ± 1 POPC (90%)	(18.5% ≥ 3000 ps)
PEPC bilayer		
Op, Oc-N-CH_3	1.65 ± 0.03 pairs/PEPC (inter)	130 ± 210
	26 ± 1% single, 74 ± 1% multiple	
	0.31 ± 0.01 pairs/PEPC (intra)	
PEPC-PEPC	0.83 ± 0.01 pairs/PEPC	1100 ± 1000*
	63 ± 1 PEPC (88%)	(10% ≥ 3000 ps)
DMPC-Chol bilayer†		
Op, Oc-N-CH_3	1.64 ± 0.04 pairs/DMPC (inter)	175 ± 250
	25 ± 3% single, 75 ± 3% multiple	
	0.25 ± 0.01 pairs/DMPC (intra)	
DMPC-DMPC	0.86 ± 0.01 pairs/DMPC	950 ± 750*
	51 ± 1 DMPC (91%)	(18.5% ≥ 2000 ps)
DMPC bilayer‡		
Op, Oc-N-CH_3	2.08 ± 0.04 pairs/DMPC (inter)	
	24 ± 3% single, 76 ± 3% multiple	
	0.28 ± 0.01 pairs/DMPC (intra)	
DMPC-DMPC	1.07 ± 0.01 pairs/DMPC	1400 ± 1000*
	67 ± 1 DMPC (93%)	(18% ≥ 3000 ps)

Numbers of charge pairs and charge-paired molecules per PC, and the lifetimes of pairing, are given (Op is a nonester phosphate and Oc is a carbonyl oxygen atom; N-CH_3 is a choline methyl group). The errors are standard errors, except for the lifetimes, where errors are standard deviations (due to the method of lifetime calculations).

*Only lower-limit estimate; see text (in parentheses, the percentage of lifetimes longer than the time window used for analysis is given).

†Pasenkiewicz-Gierula et al. (2000).

‡Pasenkiewicz-Gierula et al. (1999).

TABLE 5 Percentage of linked PC molecules, the average number PC-PC pairs linked by both types of short-distance interactions, and the average number of short-distance interactions (individual links) per PC in POPC, PEPC, DMPC-Chol, and DMPC bilayers

Bilayer	% molecules	Number of pairs/PC	Number of individual links/PC
POPC	96 ± 1	2.2 ± 0.03	4.2 ± 0.1
PEPC	96 ± 1	2.2 ± 0.03	4.4 ± 0.1
DMPC-Chol*	96 ± 1	2.2 ± 0.03	4.2 ± 0.1
DMPC†	98 ± 1	2.7 ± 0.03	5.3 ± 0.1

The errors are standard errors.

*Pasenkiewicz-Gierula et al. (2000).

†Pasenkiewicz-Gierula et al. (1999).

TABLE 6 Times (average, firm bonding, temporary break) characterizing PC-PC associations linked by both types of short-distance interactions and the number (frequency) of breaks in POPC, PEPC, DMPC-Chol, and DMPC bilayers

Bilayer	Average lifetime* (ps)	Firm bonding (ps)	Break time (ps)	Number (frequency) of breaks (1/s)
POPC	1600 ± 1200 (26% ≥ 3000 ps)	12.9	2.8	103 (0.6×10^{11})
PEPC	1400 ± 1100 (18.5% ≥ 3000 ps)	11.9	2.9	94 (0.7×10^{11})
DMPC-Chol†	1100 ± 800 (26.5% ≥ 2000 ps)	11.7	3.1	72 (0.6×10^{11})
DMPC‡	1500 ± 1200 (24% ≥ 3000 ps)	14.1	2.6	89 (0.6×10^{11})

The errors are standard deviations (due to the method of lifetimes calculations).

*Only lower-limit estimate; see text (in parentheses, the percentage of lifetimes longer than the time window used for analysis is given).

†Pasenkiewicz-Gierula et al. (2000).

‡Pasenkiewicz-Gierula et al. (1999).

compared with the DMPC bilayer. In the DMPC bilayer the number of Oc–N-CH_3 pairs per PC very slowly decreases from 2 to 1.5 when SMA increases from 40 to 85 Å^2, whereas in the POPC bilayer the number is 1 for SMA of 40 Å^2, for SMA between 45 and 70 Å^2 it decreases form 1.5 to 1, and for SMA between 70 and 90 Å^2 it gradually increases to 2 (data not shown). For the PEPC bilayer, the number of Oc–N-CH_3 pairs per PC is mostly between those for DMPC and POPC bilayers. In all three bilayers, the number of Op–N-CH_3 pairs per PC monotonously decreases from 3 to ~1, when SMA increases from 40 to 90 Å^2 (data not shown). Thus, in the POPC and PEPC bilayers the number of Op–N-CH_3 pairs depends mainly on the PC-PC distance, and that of Oc–N-CH_3 pairs depends on both the accessibility of Oc to N-CH_3 and the distance; in the DMPC

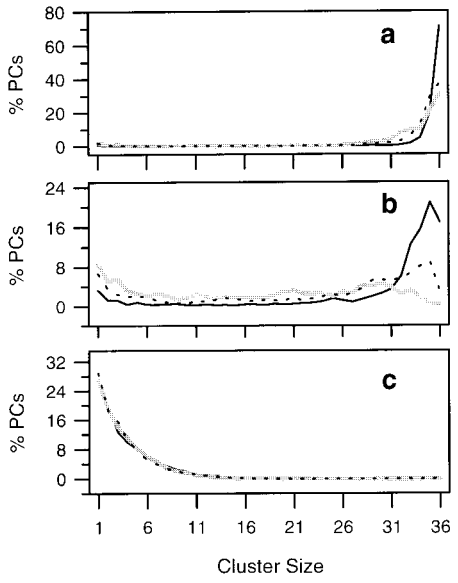

FIGURE 7 Percentage of PC molecules participating in the formation of clusters of given sizes via both mechanisms (*a*), charge pairs (*b*), and water bridges (*c*) in POPC (*dotted line*), PEPC (*gray line*), and DMPC (*solid black line*) bilayers, observed with a 1-ps time window.

bilayer, numbers of both Op–N-CH_3 and Oc–N-CH_3 pairs depend on the PC-PC distance. The difference between DMPC and POPC (PEPC) bilayers probably results from different packing of PCs with small SMA in these bilayers.

The results of the analyses described here indicate that PC molecules from different bilayers occupying the same surface area make similar numbers of H bonds with water, water bridges, and, for higher surface areas, charge pairs. These numbers are distributed about average values, as are SMAs.

For the DMPC-Chol bilayer, only an average area/PC of 69.5 $Å^2$ is available (the total surface area of the DMPC-Chol bilayer divided by the number of DMPC molecules in each leaflet of 28; Pasenkiewicz-Gierula et al., 2000), because a single molecular area for each PC and Chol molecule in the bilayer is difficult to determine. The number of H bonds with water/DMPC in this membrane, obtained from Fig. 8 *a*, is 5.2, and is the same as that obtained in direct calculation (Table 1, entries for O14, O13, O22, and O32).

DISCUSSION

In the MD simulation study, the effect of the *cis* (POPC) and *trans* (PEPC) double bond in the PC β-chain on the membrane/water interface was determined by comparing organization of the interface in mono-unsaturated POPC and PEPC membranes with that in fully saturated DMPC and DMPC-Chol membranes. In both POPC and PEPC bilayers the mean surface area per PC was 64 $Å^2$ and, in agreement with experimental results, was greater than that in the

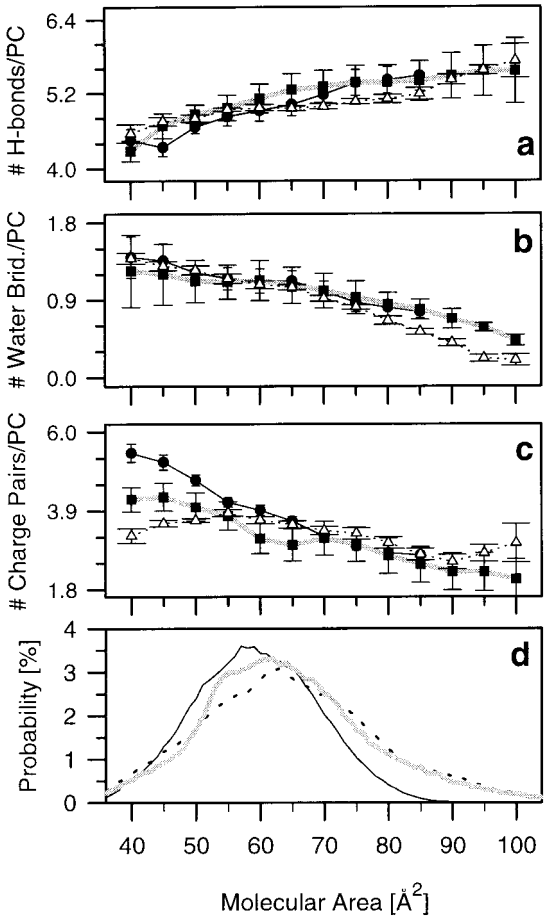

FIGURE 8 Dependence of numbers of hydrogen bonds (*a*), water bridges (*b*), and charge pairs (*c*) formed by a PC on the surface area available to the PC headgroup, in POPC (*dotted line* and *open triangles*), PEPC (*solid gray line* and *solid squares*), and DMPC (*solid black line* and *solid circles*) bilayers. Error bars are standard errors; if two values lie within the error bar the difference between these values is statistically insignificant. (*d*) Distributions of surface areas available to PC headgroups (single molecular areas) in POPC (*dotted line*), PEPC (*solid gray line*), and DMPC (*solid black line*) bilayers.

DMPC bilayer of 60 $Å^2$. As a consequence, PC hydration, measured as numbers of H bonds with water as well as H-bonded and the choline group nearest-neighbor water molecules, was greater and the number of interlipid links via water bridges and charge pairs was smaller in POPC and PEPC bilayers than in the DMPC bilayer. However, both PC hydration and the number of interlipid links were similar to those in the DMPC-Chol bilayer (Tables 1 and 2), where the average surface area per PC was 69.5 $Å^2$ (PC hydration is limited by the ability of the headgroup moieties to interact with water; thus, it shows saturation effect).

With increasing distance between PC headgroups, direct and indirect interactions among polar groups at the membrane interface become weaker and the number of interlipid links decreases. At the same time, carbonyl and, to a lesser extent, phosphate oxygen atoms become more accessible to

water, and PC hydration increases. A detailed analysis indicates that when PC-PC distance increases, intermolecular water bridges are substituted by intramolecular water bridges, whereas in the place of broken intermolecular charge pairs new H bonds between PC and water are formed. The number of intramolecular charge pairs is ~10-fold smaller than that of intermolecular ones and does not change when SMA increases from 50 to 80 Å^2.

Lipid-lipid interactions in the interfacial region of the bilayer form an extended network. This network involves 98% of PCs in the DMPC bilayer and 96% of PCs in POPC, PEPC, and DMPC-Chol bilayers. It is also more branched in the DMPC bilayer (2.7 links/PC) than in the other three bilayers (2.2 links/PC). The network of PC-PC links via water bridges has been postulated to play an important biological role in facilitating two-dimensional lateral proton diffusion on the membrane surface (Prats et al., 1987; Teissie et al., 1990). This is particularly possible because water bridges are dynamic and exchange fast (Table 3). Most likely, water molecules in clathrate-like structure around choline groups also actively participate in the lateral proton diffusion on the membrane surface.

The present study demonstrates that the membrane/water interface organization is determined by an average PC-PC distance that is directly related to the average surface area available to the PC headgroup. Correlation between a PC-PC distance and PC hydration as well as charge pairing, shown in this paper, is in accord with experimental observations (McIntosh and Simon, 1986; Slater et al. 1993; Ho et al., 1995; Yeagle et al., 1977) and other MD simulations (Lopez-Cascales et al., 1996). In this paper, a PC-PC distance was increased relative to that in the DMPC bilayer by either intercalation of Chol into the bilayer or alkyl chain lengthening and introduction of a double bond (*cis* or *trans*) to the PC β-chains. In either case, similar effects on the membrane/water interface was observed. Although this is not an unexpected result, this paper clearly shows that Chol, chain length, and a double bond, which have vastly different effects on the ordering and dynamics of the hydrocarbon chains, influence the interfacial region of the bilayer similarly, through a single quantity, which is the surface area occupied by a PC headgroup. An evidently new result of this study is that if in two bilayers built of PCs having the same headgroups but different alkyl chains, there are PCs with the same individual surface area, hydration and the number of interlipid links of these PCs are very similar (Fig. 8).

Our present MD simulation study does not show significant difference between organization of the membrane/water interface in POPC and PEPC bilayers. It is known from experimental (Subczynski et al., 1994, 1990; Pasenkiewicz-Gierula et al., 1991) and computer simulation (Pearce and Harvey, 1993) studies that certain properties of PCs with *trans* mono-unsaturated chains are similar to those of PCs with saturated chains, and different from those of PCs with *cis* mono-unsaturated chains. In another MD simulation study, we investigated the effect of the double-bond conformation on the alkyl chain region in mono-unsaturated PC and PC-Chol bilayers (Róg, 2000) and observed clear differences between PCs with *cis* and *trans* double bonds (results to be published elsewhere). They can be summarized as follow: 1) flexibility of elaidoyl chains with a *trans* double bond is greater than that of oleoyl chains and similar to that of myristoyl chains; 2) depth of water penetration of the alkyl chain region beyond carbonyl groups in the POPC bilayer is slightly smaller than in the PEPC bilayer and much smaller than in the DMPC bilayer; 3) Chol molecules are nearly uniformly distributed in DMPC-Chol and PEPC-Chol bilayers whereas they form small aggregates in the POPC-Chol bilayer. Because these three observations agree well with published data (Subczynski et al., 1994, 1990; Pasenkiewicz-Gierula et al., 1991; Pearce and Harvey, 1993) we can conclude that although conformation of the double bond (*cis* or *trans*) in the PC β-chain affects properties of the hydrocarbon chain region of the bilayer, it has little effect on the membrane/water interface.

CONCLUSIONS

Computer models of fully hydrated liquid-crystalline POPC and PEPC bilayer membranes that are stable for more than 4 ns were constructed using MD simulations. The bilayers reached thermal equilibrium after 3 and 1 ns of MD simulation, respectively.

Numbers of H bonds, and H-bonded as well as clathrated water molecules, are similar in POPC and PEPC membranes, and ~10% higher than those in the DMPC membrane. These indicate that the double-bond presence increases PC hydration whereas conformation (*cis* or *trans*) does not affect it.

In the interfacial region of POPC, PEPC, DMPC, and DMPC-Chol bilayers, headgroups of PC molecules interact via water bridges and charge pairs. In POPC, PEPC, and DMPC-Chol bilayers, the numbers of water bridges and charge pairs are ~10–20% smaller than in the DMPC bilayer. The decrease in these numbers is likely caused by an increase in the average PC-PC distance in these bilayers as compared with that in the DMPC bilayer. The number of PC-PC links is not strongly affected by the double-bond conformation (*cis* or *trans*).

In PC and PC-Chol bilayers, extended networks of intermolecular PC-PC and PC-Chol interactions are formed at the membrane/water interface. These networks involve more than 96% of lipid molecules at any instant. In POPC, PEPC, and DMPC-Chol bilayers, the networks are 20–30% less branched than that in the DMPC bilayer.

PC molecules occupying the same surface area in POPC, PEPC, and DMPC bilayers make similar numbers of H bonds with water and intermolecular PC-PC links, even though average numbers of these interactions depend on the

presence of a double bond in the PC β-chain. This illustrates a correlation between the surface area available to a PC headgroup and the number of intermolecular interactions/PC at the membrane/water interface.

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